

AMENDMENTS TO THE SPECIFICATION:

Please amend the paragraph on page 3, lines 14-15 as follows:

In a sixth fifth aspect the present invention relates to a mashing process comprising application of an acid alpha-amylase.

Please amend the paragraph on page 11, lines 4-8 as follows:

In a sixth fifth aspect of the invention an acid alpha-amylase, such as an acid alpha-amylase derived from a fungus, preferably of the genus *Aspergillus*, preferably from the species *A. niger*, and most preferably having at least 50 %, at least 60%, at least 70%, at least 80% or even at least 90% homology to the sequence shown in SEQ ID No: 1 is used in a brewing process.

Please amend the paragraph from page 12, line 34 – page 13, line 11 as follows:

Thus at temperatures in the interval 70°C to 78°C only the barley malt alpha- and beta-amylases will exhibit notable activity, and at temperatures above 78°C the endogenous enzymes activity will be negligible. In such a mashing process the added mashing enzymes will thus constitute a very essential part of or all enzyme activity. According to one embodiment of the sixth fifth aspect of the invention enzyme activities needed for the mashing process to proceed are exogenously supplied and may be added to the mash ingredients, e.g. the water or the grist before forming the mash, or it may be added during or after forming the mash. The enzymes are preferably supplied all at one time at the start of the process; however, one or more of the enzymes may be supplied at one or more times prior to, at the start, or during the process of the sixth aspect of the invention. In addition to an acid alpha-amylase (E.C. 3.2.1.1) the enzyme activities added may comprise one or more of the following activities; a protease (E.C. 3.4.), cellulase (E.C. 3.2.1.4) and a maltose generating enzyme. The maltose

generating enzyme is preferably a beta-amylase (E.C. 3.2.1.2) or even more preferably a maltogenic alpha-amylase (E.C. 3.2.1.133).

Please amend the paragraph on page 13, lines 15-24 as follows:

In accordance with the sixth fifth aspect of the invention a starch containing slurry, the mash, is obtained by mixing a grist comprising at least 5%, or preferably at least 10%, or more preferably at least 15%, even more preferably at least 25%, or most preferably at least 35%, such as at least 50%, at least 75%, at least 90% or even 100% (w/w of the grist) barley malt with water. Preferably at least 5%, preferably at least 10%, more preferably at least 20%, even more preferably at least 50%, at least 75% or even 100% of the barley malt is well modified barley malt. In one embodiment the grist comprises other malted grain than barley malt, so that at least 10%, at least 25%, preferably at least 35%, more preferably at least 50%, even more preferably at least 75%, most preferably at least 90% (w/w) of the grist is other malted grain than barley malt.

Please amend the paragraphs from page 14, line 2 – page 15, line 30 as follows:

The initial incubation temperature is preferably at least 70°C, preferably at least 71°C, more preferably at least 72°C, even more preferably at least 73°C, or most preferably at least 74°C, such as at least 75°C, at least 76°C, at least 77°C, at least 78°C, at least 79°C, at least 80°C, at least 81°C, such as at least 82°C. A preferred embodiment of the mashing process of the sixth fifth aspect of the invention includes incubating the mash at the initial incubation temperature of at least 70°C and maintaining a temperature of at least 70°C, preferably at least 71°C, more preferably at least 72°C, even more preferably at least 73°C, or most preferably at least 74°C, such as at least 75°C, at least 76°C, at least 77°C, at least 78°C, at least 79°C, at least 80°C, at least 81°C, at least 82°C, at least 83°C, at least 84°C, or at least 85°C i.e. a

temperature that never falls below 70°C for the duration of the incubation period. During the incubation period the temperature is preferably held below 100°C, such as below 99°C, 98°C, 97°C, 96°C, 95°C, 94°C, 93°C, 92°C, 91°C, or even below 90°C.

In the mashing process of the sixth fifth aspect of the invention the temperature may be held constant for the duration of the incubation, or, following a period of an essentially constant temperature (the initial incubation temperature) for the first part of the incubation the temperature may be raised, either as a slow continuously increase, or as one or more stepwise increment(s) during the incubation. Alternatively the temperature may be decreased during the incubation. In one embodiment the initial incubation temperature is at least 70°C and during the incubation the temperature is increased with at least 1°C, 2°C, 3°C, 4°C, 5°C, 6°C, 7°C, 8°C, 9°C or preferably with at least 10°C, or more preferably with at least 12°C, such as 15°C. In another embodiment the initial incubation temperature is at least 75°C, or preferably at least 80°C, and the temperature is decreased during the incubation with at least 5°C, or preferably with at least 1°C, 2°C, 3°C, 4°C, 5°C, 6°C, 7°C, 8°C, 9°C or preferably with at least 10°C, or more preferably with at least 15°C. In a particular embodiment the incubation comprises maintaining the mash at a temperature of at least 75°C, preferably at least 76°C, more preferably at least 77°C, even more preferably at least 78°C, such as at least 79°C, at least 80°C, at least 81°C, 82°C, 83°C, 84°C, 85°C, 86°C, 87°C, 88°C, 89°C or at least 90°C for a period of at least 1 minute, preferably for at least 5 minutes, more preferably for at least 15 minutes, even more preferably for at least 20 minutes, such as at least 30 minutes, at least 40 minutes, at least 50 minutes, at least 60 minutes, at least 90 minutes, or at least 120 minutes. In another particular embodiment the incubation comprises maintaining the mash at a temperature of at least 75°C, preferably at least 76°C, more preferably at least 77°C, even more preferably at least 78°C, such as at least 79°C, at least 80°C, such as at least 81°C, 82°C, 83°C, 84°C, 85°C, 86°C, 87°C, 88°C, 89°C or at least 90°C for at least 1% of the total incubation time, preferably for at least 5%, more preferably for at least 15%, even more preferably for at least 20%, or at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, such as for 100% of the total incubation time. The duration of the incubation is

preferably at least 15 minutes, typically between 30 minutes and 2 ½ hours, e.g. at least 45 minutes, at least 1 hour, at least 1 ¼ hour, at least 1 ½ hour, at least 1 ¾ hour or at least 2 hours.

In the mashing process of the sixth fifth aspect of the invention the grist may in addition to barley malt preferably comprise adjunct such as unmalted barley, or other malted or unmalted grain, such as wheat, rye, oat, corn, rice, milo, millet and/or sorghum, or raw and/or refined starch and/or sugar containing material derived from plants like wheat, rye, oat, corn, rice, milo, millet, sorghum, potato, sweet potato, cassava, tapioca, sago, banana, sugar beet and/or sugar cane. For the invention adjuncts may be obtained from tubers, roots, stems, leaves, legumes, cereals and/or whole grain. Preferably the adjunct to be added to the mash of the sixth fifth aspect of the invention has gelatinization temperatures at or below the process temperature. If adjuncts such as rice or corn, or other adjuncts with similar high gelatinization temperature, are to be used in the process of the sixth fifth aspect of the invention, they may preferably be cooked separately to ensure gelatinization before being added to the mash of the sixth fifth aspect of the invention, or the gelatinized adjunct starch may be mashed separately from the mash adding appropriate enzymes to ensure saccharification before being added to the mash. Methods for gelatinization and saccharification of brewing adjuncts are well known in the arts. Adjunct comprising readily fermentable carbohydrates such as sugars or syrups may be added to the barley malt mash before, during or after mashing process of the sixth fifth aspect of the invention but is preferably added after the mashing process. Preferably a part of the adjunct is treated with a protease and/or a beta-glucanase before being added to the mash of the sixth fifth aspect of the invention. During the mashing process, starch extracted from the grist is gradually hydrolyzed into fermentable sugars and smaller dextrins. Preferably the mash is starch negative to iodine testing, before extracting the wort.

Following the mashing step of the sixth fifth aspect of the invention obtaining the wort from the mash typically includes straining the wort from the spent grains, i.e. the

insoluble grain and husk material forming part of grist. Hot water may be run through the spent grains to rinse out, or sparge, any remaining extract from the grist.

Please amend the paragraph from page 15, line 33 – page 16, line 3 as follows:

The wort produced by the mashing process of the sixth fifth aspect of the invention may be fermented to produce a beer. Fermentation of the wort may include pitching the wort with a yeast slurry comprising fresh yeast, i.e. yeast not previously used for the invention or the yeast may be recycled yeast. The yeast applied may be any yeast suitable for beer brewing, especially yeasts selected from *Saccharomyces* spp. such as *S. cerevisiae* and *S. uvarum*, including natural or artificially produced variants of these organisms. The methods for fermentation of wort for production of beer are well known to the person skilled in the arts.

Please amend the paragraph on page 16, lines 7-12 as follows:

The enzymes to be applied in the sixth fifth aspect of present invention should be selected for their ability to retain sufficient activity at elevated temperatures, such as at the process temperature of the processes, as well as for their ability to retain sufficient activity under the moderately acid pH regime in the mash and should be added in effective amounts. The enzymes may be derived from any source, preferably from a plant or an algae, and more preferably from a microorganism, such as from a bacteria or a fungi.

Please amend the paragraph on page 17, lines 8-12 as follows:

A particular cellulase to be used in the processes of the sixth fifth aspect of the invention may be an endo-glucanase, such as an endo-1,4-beta-glucanase. Contemplated are beta-glucanases having at least 90% homology to amino acid sequence disclosed as SEQ.ID.NO:1 in Danish patent application PA2002 00130, such

as at least 92%, at least 95%, at least 96%, at least 97%, at least 98%, or particularly at least 99%.

Please amend the paragraphs from page 17, line 20 – page 18, line 7 as follows:

A particular alpha-amylase (EC 3.2.1.1) to be used in the processes of the sixth fifth aspect of the invention may be any fungal alpha-amylase, preferably an acid alpha-amylase. Preferably the acid alpha-amylase is derived from a fungus of the genus *Aspergillus*, preferably from the species *A. niger*, and most preferably having at least 50 %, at least 60%, at least 70%, at least 80% or even at least 90% homology to the sequence shown in SEQ ID No:1 is used in a brewing process. Fungal alpha-amylases may be added in an amount of 1-1000 AFAU/kg DM, preferably from 2-500 AFAU/kg DM, preferably 20-100. AFAU/kg DM.

Another acid alpha-amylase enzyme to be used in the processes of the sixth fifth aspect of the invention may be a *Bacillus* alpha-amylase. Well-known *Bacillus* alpha-amylases include alpha-amylase derived from a strain of *B. licheniformis*, *B. amyloliquefaciens*, and *B. stearothermophilus*. Other *Bacillus* alpha-amylases include alpha-amylase derived from a strain of the *Bacillus* sp. NCIB 12289, NCIB 12512, NCIB 12513 or DSM 9375, all of which are described in detail in WO95/26397, and the alpha-amylase described by Tsukamoto et al., Biochemical and Biophysical Research Communications, 151 (1988), pp. 25-31. In the context of the present invention a contemplated *Bacillus* alpha-amylase is an alpha-amylase as defined in WO99/19467 on page 3, line 18 to page 6, line 27. A preferred alpha-amylase has an amino acid sequence having at least 90% homology to SEQ ID NO: 4 in WO99/19467, such as at least 92%, at least 95%, at least 96%, at least 97%, at least 98%, or particularly at least 99%. Most preferred variants of the maltogenic alpha-amylase comprise the variants disclosed in WO99/43794. Contemplated variants and hybrids are described in WO96/23874, WO97/41213, and WO99/19467. Specifically contemplated is a recombinant *B. stearothermophilus* alpha-amylase variant with the mutations; I181* +

G182* + N193F. *Bacillus* alpha-amylases may be added in the amounts of 1.0-1000 NU/kg dm, preferably from 2.0-500 NU/kg dm, preferably 10-200 NU/kg dm.

Please amend the paragraph on page 18, lines 10-22 as follows:

A particular enzyme to be used in the processes of the sixth fifth aspect of the invention is a maltogenic alpha-amylase (E.C. 3.2.1.133). Maltogenic alpha-amylases (glucan 1,4-alpha-maltohydrolase) are able to hydrolyse amylose and amylopectin to maltose in the alpha-configuration. Furthermore, a maltogenic alpha-amylase is able to hydrolyse maltotriose as well as cyclodextrin. Specifically contemplated maltogenic alpha-amylases may be derived from *Bacillus* sp., preferably from *Bacillus stearothermophilus*, most preferably from *Bacillus stearothermophilus* C599 such as the one described in EP 120.693. This particular maltogenic alpha-amylase has the amino acid sequence shown as amino acids 1-686 of SEQ ID NO: 1 in US 6,162,628. A preferred maltogenic alpha-amylase has an amino acid sequence having at least 90% homology to amino acids 1-686 of SEQ ID NO:1 in US 6,162,628 preferably at least 92%, at least 95%, at least 96%, at least 97%, at least 98%, or particularly at least 99%. Most preferred variants of the maltogenic alpha-amylase comprise the variants disclosed in WO99/43794.

Please amend the paragraph on page 18, lines 25-26 as follows:

Another particular enzyme to be used in the processes of the sixth fifth aspect of the invention may be a beta-amylase (E.C 3.2.1.2).

Please amend the paragraphs from page 18, line 35 – page 19, line 12 as follows:

A further particular enzyme to be used in the processes of the sixth fifth aspect of the invention may be a glucoamylase (E.C.3.2.1.3) derived from a microorganism or

a plant. Preferred are glucoamylases of fungal or bacterial origin selected from the group consisting of *Aspergillus* glucoamylases, in particular *A. niger* G1 or G2 glucoamylase (Boel et al. (1984), EMBO J. 3 (5), p. 1097-1102), or variants thereof, such as disclosed in WO92/00381 and WO00/04136; the *A. awamori* glucoamylase (WO84/02921), *A. oryzae* (Agric. Biol. Chem. (1991), 55 (4), p. 941-949), or variants or fragments thereof. Glucoamylases may be added in effective amounts well known to the person skilled in the art.

Another enzyme of the process of the sixth fifth aspect of the present invention may be a debranching enzyme, such as an isoamylase (E.C. 3.2.1.68) or a pullulanases (E.C. 3.2.1.41). Isoamylase hydrolyses alpha-1,6-D-glucosidic branch linkages in amylopectin and beta-limit dextrins and can be distinguished from pullulanases by the inability of isoamylase to attack pullulan, and by the limited action on alpha-limit dextrins. Debranching enzyme may be added in effective amounts well known to the person skilled in the art.